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Review

Anti-Neutrophil Cytoplasmic Antibody Pathogenesis in Small-Vessel Vasculitis

An Update

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Vasculitides associated with serum positivity for anti-neutrophil cytoplasmic antibodies (ANCAs) that affect small- to medium-sized vessels are commonly known as ANCA-associated vasculitis (AAV) and include Wegener's granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome. Evidence derived from both *in vitro* studies and recent animal models points to a pathogenic role of ANCAs in AAV. In 2002, the first *in vivo* breakthrough in the pathogenesis of ANCAs showed that mouse ANCAs against myeloperoxidase (MPO) led to intrinsic pauci-immune renal vasculitis in mice. In 2004, a report using both *in vitro* and *in vivo* studies proposed that proteinase 3 (PR3)-directed autoimmunity involved the complementary peptide of PR3 (cPR3), which is encoded by the antisense strand of the PR3 gene. The last breakthrough came in October 2008 with a previously undescribed molecular explanation for the origin and development of injury in pauci-immune renal vasculitis, with potential clinical implications. This report showed that infection by fimbriated bacteria may trigger cross-reactive autoimmunity to a previously characterized ANCA antigen, lysosomal membrane protein-2, which is contained in the same vesicles that harbor MPO and PR3. Infection by fimbriated bacteria resulted in the production of autoantibodies, which activated neutrophils and killed human microvascular endothelium *in vitro* and caused renal vasculitis in rats. Although the evidence for a pathogenic role of ANCAs, mainly MPO-ANCAs, is striking, various questions remain unanswered. Understanding the key pathogenic mechanisms of AAV may provide a safer, more rational therapeutic approach than the traditional (ie, corticosteroids and immunosuppressants) treatment strategy. *(Am J Pathol 2009, 175:1790–1798; DOI: 10.2353/ajpath.2009.090533)*

Anti-neutrophil cytoplasmic antibodies (ANCAs) were discovered by chance in 1982 when Davies et al[1] were studying antinuclear antibodies in serum samples from patients with segmental necrotizing glomerulonephritis. Using indirect immunofluorescence applied to neutrophils, a diffuse cytoplasmic, but not nuclear, staining pattern was observed. In 1985, van der Woude et al[2] found that cytoplasmic ANCAs occurred mainly in patients with Wegener's granulomatosis (WG), and interest in ANCAs skyrocketed. In 1988,[3] a distinct perinuclear pattern in serum samples from patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis was reported. Enzyme-linked immunosorbent assay showed that myeloperoxidase (MPO) was the chief antigenic target of perinuclear ANCAs. Two years later, proteinase 3 (PR3) was recognized as the major autoantigen accounting for the cytoplasmic ANCA pattern of WG.[4,5]

The vasculitides are often serious and sometimes fatal diseases that require prompt recognition and treatment. Symptomatic involvement of affected organs may occur in isolation or in combination with multiple organ involvement. Vasculitic syndromes are normally categorized by the type and predominant size of the blood vessels most commonly affected (Table 1).[6,7] The distribution of affected organs may suggest a particular vasculitic disorder, but there is significant overlap.

ANCA-associated small-vessel vasculitis should be suspected in any patient presenting with multisystemic

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Table 1. Classification of Vasculitis

Large-vessel vasculitis	Medium-sized vessel vasculitis	Small-vessel vasculitis
Takayasu arteritis	Polyarteritis nodosa	ANCA-related vasculitis
Giant cell arteritis	Kawasaki disease	Churg-Strauss syndrome
	Isolated central nervous system vasculitis	Wegener's granulomatosis
		Microscopic polyangiitis
		Drug-induced ANCA-associated vasculitis
		Henoch-Schönlein purpura
		Essential cryoglobulinemic vasculitis
		Hypersensitivity vasculitis
		Hypersensitivity vasculitis
		Vasculitis due to connective tissue disorders
		Vasculitis due to viral infection
		Paraneoplastic small-vessel vasculitis

Modified from Jennette and Falk.[7]

disease not caused by infectious or malignant processes (eg, renal failure, skin rashes, pulmonary infiltrates, or neurological manifestations such as peripheral neuropathy). Constitutional symptoms are also common.[6–8] Renal involvement in vasculitis may progress to renal failure and renal biopsy commonly reveals glomerulonephritis. Although renal-limited vasculitis is closely associated with ANCAs, WG, microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS) are systemic forms of ANCA-associated vasculitis (AAV) with common extrarenal involvement.

Vasculitides associated with serum positivity for ANCAs that affect small to medium-sized vessels are commonly known as AAV. Focal necrosis, crescentic formation, and the absence or paucity of immunoglobulin deposits characterize glomerulonephritis in patients with AAV. Lung involvement ranges from fleeting focal infiltrates or interstitial disease to massive pulmonary hemorrhagic alveolar capillaritis, the most life-threatening manifestation of small-vessel vasculitis.[7] ANCAs directed to proteinase 3 (PR3-ANCAs) are detected mainly in WG, whereas anti-myeloperoxidase antibodies (MPO-ANCAs) are predominantly found in MPA and CSS.

Vasculitis Classification

Classification criteria for most of the major forms of vasculitis were established by the American College of Rheumatology in 1990[9] and were based on prospective data from patients with vasculitis; they do not include all characteristics of a particular disorder, only those that help distinguish it from other vasculitides. The criteria were revisited in 1994 at the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis, at which the concept of MPA was firmly established.

WG predominantly affects the upper and lower respiratory tracts and the kidneys, in which it may lead to rapidly progressive glomerulonephritis as a result of necrotizing and crescentic glomerulonephritis. In the lungs, WG can cause life-threatening diffuse alveolar hemorrhage as a result of (pauci-immune) alveolar necrotizing capillaritis.[10] Localized forms of WG are usually limited to the eyes, ears, nose, and lungs. Histologically, WG is characterized by granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small- to medium-sized vessels (eg, capillaries, venules, arterioles, and arteries).[8]

CSS is characterized by asthma, hypereosinophilia, and transient pulmonary infiltrates. Rapidly progressive glomerulonephritis and pulmonary hemorrhage are less common than in MPA and WG. The typical histopathological features of CSS include eosinophil-rich, granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small- to medium-sized vessels and associated with asthma and eosinophilia.[8,10] The natural history of CSS is characterized by three clinical stages: a prodromic phase consisting of severe asthma (99% of patients[11]), followed by eosinophil infiltration into tissues (eg, pulmonary infiltrates), and, last, MPO-ANCA-associated systemic vasculitis affecting mainly the skin, kidneys, and peripheral nerves in addition to asthma and peripheral eosinophilia (3 to 4 years after onset).[12] There may be granulomatous disease in the third stage.[13] However, the three stages do not have to follow each other. Studies of ANCA prevalence and disease patterns show two general subsets of patients depending on ANCA positivity: one with ANCAs and a predominance of histology-proven necrotizing small-vessel vasculitis and another without ANCAs and with a higher incidence of eosinophil infiltration of the lung, heart, and gastrointestinal tract.[14] A recent study linked *HLA-DRB4* with CSS and an increased risk of vasculitic manifestations.[15]

MPA is characterized by pauci-immune necrotizing small-vessel vasculitis without granuloma formation, with or without involvement of medium-sized arteries. The clinical spectrum is similar to WG, although ear, nose, throat, and lung involvement is less common[10] and renal involvement may be the only manifestation. About half the patients with MPA develop necrotizing alveolar capillaritis-induced pulmonary hemorrhage. MPA is the most common cause of pulmonary-renal syndrome (Figure 1). Histologically, MPA is characterized by necrotizing vasculitis with few or no immune deposits affecting small vessels (capillaries, venules, and arterioles). There may be necrotizing arteritis involving small- and medium-sized arteries.[8]

Currently, immunosuppressants combined with glucocorticoids are the mainstay of AAV treatment, including renal-limited vasculitis. Although dramatically improving survival, 25% of patients have severe treatment-related adverse events and the 5-year relapse rate is 50% such that AAV becomes a chronic, relapsing disorder with accumulative, irreversible organ damage. Repeated disease episodes then lead to intensification of toxic immunosuppressants. Understanding the key pathogenic mechanisms of AAV may provide a safer, more rational therapeutic approach.

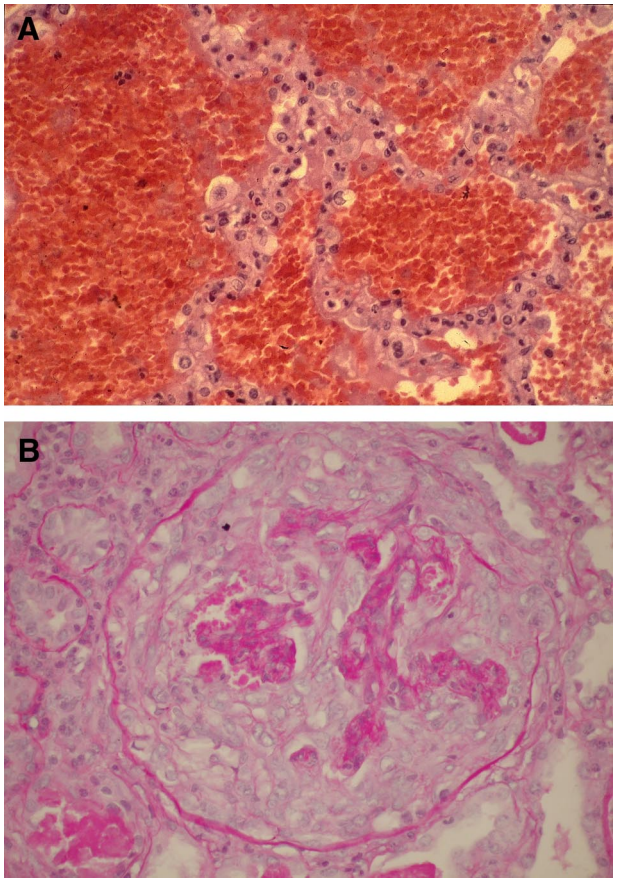

Figure 1. Histological hallmarks of ANCA-associated vasculitis. **A:** A lung biopsy specimen shows severe alveolar capillaritis with alveolar hemorrhage. Observe the thickened interalveolar septum with infiltrates of mononuclear cells and some neutrophils (H&E stain; original magnification ×60). **B:** Renal biopsy specimen showing necrotizing and crescentic glomerulonephritis in a patient with MPA. Note the crescentic formation and glomerular capillary necrosis (H&E; original magnification, ×400).

Pathogenesis: Animal Models

A pathogenic role for ANCAs has always been suspected because of their association with small-vessel vasculitis. Numerous animal models reinforce the theoretical pathogenicity of ANCAs (Table 2).[14,16–24] For example, Xiao et al[17] immunized MPO-knockout mice with murine MPO. When MPO-immunized splenocytes were transferred to mice lacking B-functioning and T-functioning lymphocytes ($Rag2^{-/-}$), MPO-ANCAs developed in a dose-dependent manner. Mice receiving the largest amount of MPO-immunized splenocytes developed severe necrotizing and crescentic glomerulonephritis and systemic vasculitis, including pulmonary capillaritis. However, all mice receiving the highest splenocyte dose developed nonsevere immune complex-mediated glomerulonephritis. In addition, the researchers injected MPO-ANCAs into $Rag2^{-/-}$ and wild-type mice to trigger anti-idiotype antibodies, which react with the original autoantigen. Both strains presented focal necrotizing and crescentic glomerulonephritis without immune complexes. The authors concluded that MPO-ANCAs intrinsically produced pauci-immune necrotizing and crescentic glomerulonephritis. However, because renal lesions in $Rag2^{-/-}$ mice receiving MPO-ANCAs were not as widespread as those seen in $Rag2^{-/-}$ mice given MPO-immunized splenocytes, other factors (eg, T lymphocytes and low-level immune complex deposition) might enhance the inflammatory process.

Although ANCA-associated vasculitides do not, by definition, have immune complexes, immune deposits were shown by electron microscopy in 50% of renal biopsy specimens from patients with ANCA-positive necrotizing and crescentic glomerulonephritis, necrotizing arteritis, or both.[26] The addition of bacterial lipopolysaccharide increased the number of glomeruli affected and augmented tumor necrosis factor-α (TNF-α) levels.[19] This result seems to confirm *in vitro* hypotheses underlining the importance of synergistic TNF-α priming for effective neutrophil activation and suggests how infection might exacerbate the effects of ANCAs. The role of Toll-like receptors in neutrophil priming also deserves investigation. Subsequent experiments in mice have demonstrated an essential role for both neutrophils[20] and the alternative complement pathway in AAV.[21,23]

In 2005, Little et al[16] developed a Wistar-Kyoto rat model in which focal necrotizing glomerulonephritis (FNGN) and pulmonary capillaritis were induced after immunization with purified human MPO, and the effects of MPO-ANCAs on the induction of leukocyte-endothelium interactions were explored using intravital microscopy of mesenteric venules. Administration of the chemokine CXCL-1 (a rat homolog of interleukin-8) in the mesenterium of both immunized and naïve rats, which received purified IgG from sera of MPO-immunized rats, led to increased leukocyte adherence and transmigration, with microvasculature focal hemorrhage at chemokine application sites. This experiment not only reinforced the pathogenic effect of ANCAs but also confirmed *in vitro* flow models showing that ANCAs in collaboration with a synergistic proinflammatory stimulus promote neutrophil adhesion to the endothelium *in vivo*. In contrast to accumulated evidence from MPO-ANCA animal models, there is no convincing *in vivo* evidence of PR3-ANCA pathogenicity.

Recently, Kain et al[25] immunized rats with rabbit immunoglobulin specific to human lysosomal membrane protein-2 (LAMP-2), which cross-reacts with rat LAMP-2. All rats developed severe renal injury; 22% of glomeruli exhibited focal capillary necrosis after 24 hours, and 21% of glomeruli developed crescents within 48 hours. Control rats and rats injected with nonspecific rabbit immunoglobulin developed no lesions. Incubation with a monoclonal antibody to human LAMP-2 caused activation of neutrophils and induced apoptosis of endothelial cells.

Little et al[16] also created an experimental animal model of MPO-ANCA-induced renal and pulmonary damage and found the precise amount of MPO needed to cause the lesions in all, not only some, animals. Previous reports showed heterogeneity in the number of affected glomeruli, which, Little et al speculated, could be explained by the different concentrations of MPO used. The authors found that the addition of an adjuvant allowed the amount of MPO required to produce similar renal and

Table 2. Animal Models of ANCA-Associated Vasculitis

Author and reference	Hypothesis tested	Experimental model	Main results
Little et al[16]	MPO-ANCAs are able to promote leukocyte-endothelium interactions *in vivo*	Wistar-Kyoto rats were immunized with human MPO and developed human anti-MPO that cross-reacted with murine MPO IgG from immunized rats was passively transferred to nonimmunized rats CXCL-1 was applied to the mesenterium of rats	Rats immunized with human MPO developed NCGN and capillaritis Leukocyte adherence and transmigration with microvasculature focal hemorrhage was observed (by intravital microscopy) in both immunized and nonimmunized rats in places where CXCL-1 was applied
Xiao et al[17]	MPO-ANCAs are pathogenic	Passive administration of anti-MPO IgG (derived from the immunization of MPO knockout mice with murine MPO) to mice without functioning T or B lymphocytes (Rag2$^{-/-}$) and to wild-type B6 mice	Rag 2$^{-/-}$ mice developed focal NCGN without immune deposits with approximately 15% of glomeruli suffering damage
Pfister et al[18]	PR3-ANCAs are pathogenic	Passive transfer of PR3-ANCA-containing IgG (obtained from immunization of mice lacking PR3 and elastase with mouse PR3) to wild-type mice in the presence of LPS	No development of human AAV features
Huugen et al[19]	A proinflammatory stimulus of infectious origin would aggravate MPO-ANCA-dependent damage	Wild-type B6 mice were transferred with IgG MPO-ANCAs and also treated with bacterial LPS	Dose-dependant increase of the glomerular damage
Xiao et al[20]	Neutrophils are key-effector cells in the pathogenesis of MPO-ANCA-induced NCGN	IgG MPO-ANCAs were transferred to wild-type B6 mice Neutrophils were depleted using NIMP-R14 rat monoclonal antibodies	Neutrophil depletion prevented MPO-ANCA IgG-related NCGN
Xiao et al[21]	Complement plays a role in AAV pathogenesis	Wild-type B6 mice were administered IgG MPO-ANCAs and Rag2$^{-/-}$ mice received anti-MPO splenocytes Complement was depleted using cobra venom factor C5, C4, and factor B knockout mice were also administered IgG MPO-ANCAs	Complement depletion totally blocked NCGN in all mice IgG MPO-ANCAs caused disease in wild-type B6 and C4 mice but not in C5$^{-/-}$ and factor B$^{-/-}$ mice, suggesting involvement of the alternative complement pathway
Ruth[22]	Both anti MPO-CD4$^+$ T lymphocytes (cellular response) and MPO-ANCAs play a role in NCGN	Wild-type B6, MPO$^{-/-}$, and μMT$^{-/-}$ (mice lacking mature B cells) were immunized with human MPO and developed both humoral (except μMT$^{-/-}$ mice) and cellular autoimmunity to mice MPO Sheep anti-mouse GMB antibodies were used to induce neutrophil recruitment to the glomeruli Anti-CD4$^+$ monoclonal antibodies were employed to neutralize CD4$^+$ T cells	Immunized wild-type B6 mice presented accumulation of neutrophils, CD4$^+$ cells, macrophages, and crescent formation after injection of anti-GBM antibodies Administration of anti-CD4 antibodies to immunized wild-type mice prevented crescent formation and recruitment of macrophages and leukocytes but not neutrophils
Huugen et al[23]	Inhibition of C5 neutralizes MPO-ANCA-related damage	Wild-type B6 mice were injected with IgG MPO-ANCAs, LPS, and antimurine C5 monoclonal antibody BB5.1	Anti-C5 pretreatment prevented NCGN induced by Ig MPO-ANCAs and LPS Anti-C5 treatment strongly attenuated NCGN induced by Ig MPO-ANCAs and LPS
Kessenbrock et al[24]	ANCA-mediated activation induces NETs formation	Primed neutrophils with TNF-α and incubated them with purified IgG from individuals with small-vessel vasculitis	NET formation in neutrophils incubated with ANCA-IgG Induction of NETs with PR3 mouse monoclonal antibody
Kain et al[25]	Characterized autoantibodies to LAMP-2 and showed that they are a new ANCA subtype in almost all individuals	Immunized rats with rabbit immunoglobulin against human LAMP-2, which cross-reacts with rat LAMP-2 Injected 15 Wistar-Kyoto rats i.v. with human LAMP-2 rabbit IgG that cross-reacts LAMP-2 with rat	All rats developed severe renal injury 22% of glomeruli exhibited focal capillary necrosis after 24 hours, which was present in 21% of glomeruli developed crescents

NCGN, necrotizing and crescentic glomerulonephritis; LPS, lipopolysaccharide; GMB, glomerular basement membrane; NET, neutrophil extracellular trap.

lung effects to be reduced.[16] In addition, the study by Little et al could result in a clear reduction of the number of animals required to demonstrate a statistical effect of an agent, as the authors found that all animals developed renal and pulmonary damage, whereas previous studies revealed variability and diverse disease severity.

Role of Cell Interactions in Vascular Injury

Role of Neutrophils

Since 1990, when Falk et al[27] showed that ANCAs can stimulate neutrophils to undergo a respiratory burst and

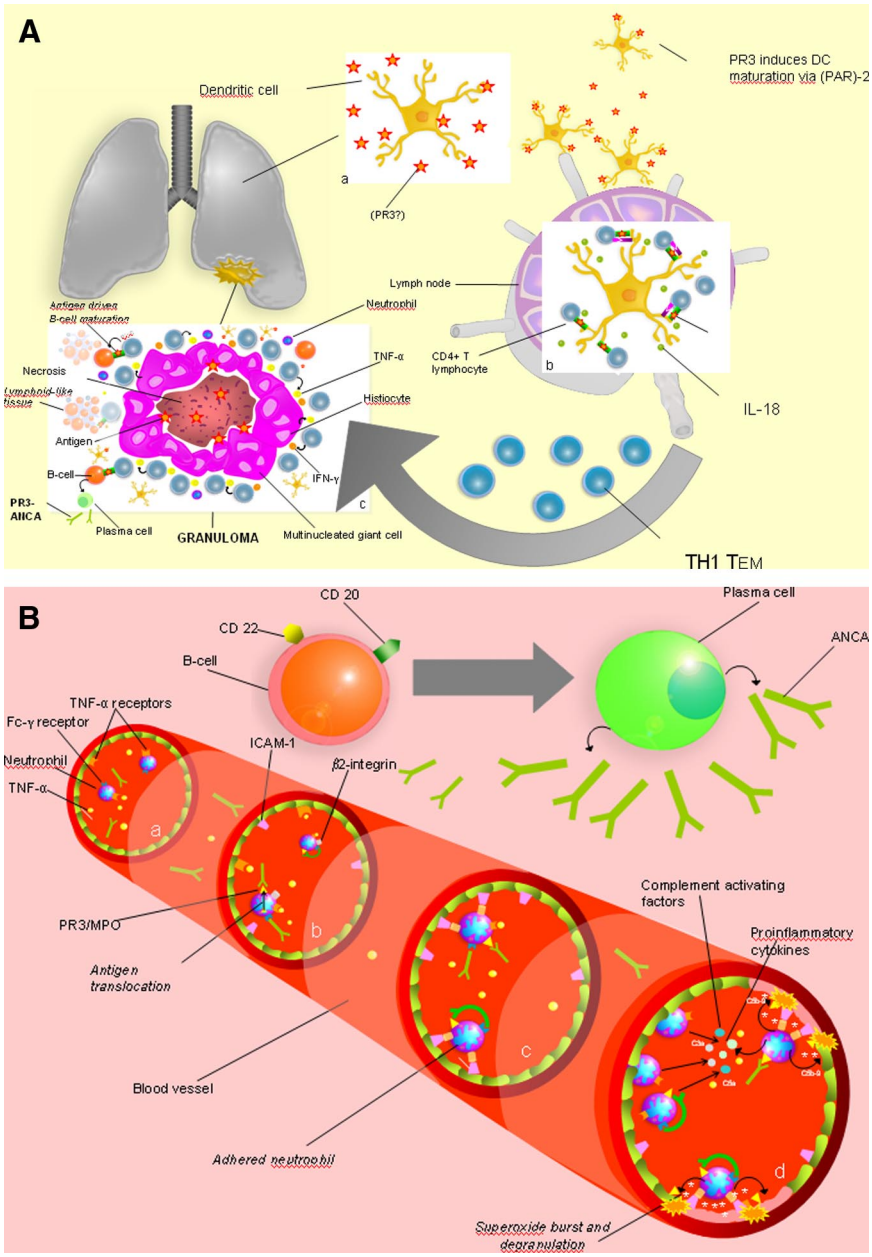

Figure 2. ANCA-associated vasculitis pathogenic model. **A:** Granulomatous inflammation in WG. **a:** Lung DCs are exposed to an undefined antigen (perhaps PR3).[28] **b:** Antigen-loaded DCs travel to peripheral lymph nodes and present the antigen to naïve $CD4^+$ T lymphocytes. DCs produce interleukin-18 (IL-18), among other cytokines, and skew T cells to a Th1 phenotype. **c:** Activated Th1 effector memory T cells (TEMs) return to the lungs, where the antigen persists. TEMs secrete large amounts of interferon-γ (IFN-γ) and TNF-α, which induce macrophage recruitment and maturation and eventually lead to granulomata formation and tissue destruction. Chronic T-cell activation might promote neogenesis of lymphoid-like tissue, where affinity maturation of autoreactive B cells and plasma cells takes place (the latter secreting PR3-ANCA, which will reach the bloodstream causing vasculitis).[29] (PAR)-2, protease-activated receptor. **B:** ANCA-induced necrotizing vasculitis in WG, MPA, and CSS. **a:** TNF-α induces "priming" of neutrophils and endothelial cells. **b:** Cytokine priming allows PR3 and MPO to travel from azurophilic granules to the neutrophil surface and interact with ANCAs. In addition, TNF-α induces enhanced expression of adhesion molecules by endothelial cells. **c:** Simultaneous interaction of ANCA with their antigen and Fc-γ receptor prompts several effector functions including firm adhesion (and not rolling) of neutrophils to the cytokine-primed endothelium. **d:** Once adhered, ANCAs induce neutrophil respiratory burst and degranulation which, ultimately, cause vasculitic damage. ANCAs also promote neutrophil secretion of proinflammatory cytokines and activating complement factors, leading to the recruitment of more inflammatory cells and amplifying and perpetuating the process. ICAM-1, intercellular adhesion molecule-1.

release primary granule constituents, many *in vitro* studies have shown that ANCAs might cause *in vivo* vascular damage by inducing a wide range of neutrophil effector functions such as cytokine and chemokine release and increased adhesion to cultured endothelial cells, with their eventual lysis (Figure 2).[28–30]

Conversely, some researchers suggested that a dysfunction in neutrophil apoptosis might lead to ANCA generation. When neutrophils undergo apoptosis, primary granule constituents translocate to the cell surface.[31,32] In two experiments,[33,34] injection of apoptotic neutrophils into rodents resulted in the presence of ANCAs. Uptake of apoptotic cells is typically undertaken by macrophages and also, under certain conditions, by dendritic cells (DCs), leading to cross-presentation of self-antigens, activation of specific T lymphocytes and, ultimately, to autoimmunity.[35] Clayton et al[36] showed that immature DCs engulfed human apoptotic neutrophils; however, they observed a decrease in T-lymphocyte proliferation. The addition of TNF-α counteracted this suppressor effect. Therefore, a second signal may be needed for cross-presentation of self-antigens for ANCAs to appear. The presence of TNF-α during the influenza-like symptoms heralding ANCA-associated vasculitides might be that signal.[35] However, if apoptotic neutrophils were the triggering factor of ANCA production, patients with AAV would be expected to have antibodies against a range of antigens (not just PR3-ANCAs or MPO-ANCAs), because all granule constituents are available on apoptotic cells.

Binding of ANCAs causes neutrophil activation, with subsequent increased adhesion and migration to endo-

thelium, release of proteolytic granule enzymes (including MPO and PR3) and proinflammatory cytokines, generation of a respiratory burst, and, eventually, endothelial cell damage. Proinflammatory cytokines secreted by neutrophils as a result of ANCA binding include interleukin-1β, TNF-α, interleukin-6, interleukin-8, monocyte chemoattractant protein-1, and leukotriene B_4. ANCA-mediated cytokine secretion activates and recruits extra-inflammatory cells, amplifying and perpetuating the inflammatory response, with monocytes and T cells participating later in the process.[35,37,38]

Both ANCA F(ab′)2 and Fc engagements are needed to allow for effective neutrophil activation. β2 integrin (CD11b/CD18) might cooperate with the Fc-γ receptor to propagate the signal. ANCA-induced intracellular signal transduction pathways differ according to whether the signal is initiated by the F(ab′)2 or Fc portion of the autoantibody.[39] The ANCA IgG F(ab′)2 fragment can activate inhibitory G proteins and RAS p21 protein activator but not tyrosine kinases (sarcoma virus kinases, Syk, phosphatidylinositol-3 kinase, protein kinase B, and protein kinase C), whose activation probably relies on Fc-γ receptor binding.[39,40] Tyrosine kinase pathways are thought to induce respiratory burst through activation of NADH oxidase. Inhibitory G protein and tyrosine kinase pathways may cooperate in oxidative burst generation because they converge on the GTPase RAS p21 protein activator.[40] Indeed, transient enhanced RAS p21 protein activator activity is reported to precede a rise in superoxide production.[41]

ANCA-mediated neutrophil activation disrupts apoptosis by delaying apoptotic neutrophil surface phosphatidylserine expression, which is necessary for macrophage-mediated cell removal. Because of their delayed clearance, neutrophils then undergo secondary necrosis with subsequent release of inflammatory mediators, amplifying the process.[17] Furthermore, ANCA-opsonized apoptotic neutrophils enhance both the phagocytotic activity of macrophages and their production of proinflammatory cytokines.[19] In contrast, Abdel-Salam et al[42] detected a low affinity of PR3-ANCAs for their antigen, challenging the activating role of ANCAs, as only high concentrations of ANCAs permitted their binding. However, these results do not exclude the possibility that large amounts of ANCAs could interact with primed neutrophils at the capillary lumen and thus exert their pathogenic effects.[43] ANCAs can also induce polymerization of the actin cytoskeleton, strengthening neutrophil rigidity, which may contribute to their sequestration in capillaries. This finding may explain why ANCA-associated vasculitides have a predilection for the small vessels.[44]

Another recent report[24] sheds further light on the pathogenic mechanisms of ANCAs and neutrophils in vasculitides. In normal conditions, neutrophils release neutrophil extracellular traps (NETs), chromatin fibers that can ensnare bacteria. In AAV, it has been shown that these NETs express ANCA autoantigens, accumulate in affected kidneys, and promote the autoimmune response against neutrophils. In fact, kidney biopsies from patients with AAV confirmed the presence of neutrophils and NETs near deteriorating capillaries. Of interest, although the authors found that these NETs are also produced in the absence of infection, the infectious link is not missed in this article: the authors suggested that the propensity of neutrophils to produce NETs in these patients could be further enhanced by *Staphylococcus aureus* infection, which per se is a strong inductor of NETs and also seems to be involved in WG relapses.

Role of Effector T Cells

T cells may play a major role in ANCA-associated vasculitides. Supportive data include the following: i) ANCAs are high-affinity, class-switched antibodies and their generation necessarily relies on T cells; ii) T cells accumulate in the kidney and their number correlates with renal impairment; iii) T cells in patients with AAV react to PR3 and MPO in proliferation assays[35]; and iv) T-cell reactivity markers (eg, cytotoxic T lymphocyte-associated antigen 4) are increased in active disease.[45]

Indeed, effector T cells can accompany ANCAs and thereby amplify vascular damage. In fact, T cells are frequently found in biopsies of vasculitic areas, and some T cell-targeted therapies have reversed vasculitic manifestations.[46] Marinaki et al[47] found an association between persistent $CD4^+$ T-cell activation and disease severity in both WG and MPA. In addition, a recent animal model of MPO-ANCA-induced FNGN has provided a mechanistic basis for $CD4^+$ T cells in this disease.[22] The authors speculated on the existence of a synergistic combination of both humoral and cellular autoimmunity to MPO. Initially, ANCA induces glomerular neutrophil infiltration and degranulation with MPO release. Subsequently, MPO-specific $CD4^+$ T cells are activated and, together with macrophages, exacerbate the pathological condition.

In CSS, activation of Th1/Th2 lymphocytes, eosinophils (whose levels correlate with disease activity), the release of toxic products, and MPO-ANCAs are therefore the main pathogenic factors and apparently predominate in different phenotypes of the disease. It has been shown that, in CSS, T cells may undergo oligoclonal expansion triggered by a limited number of (probably inhaled) antigens.[11,46] The allergic background and hypereosinophilia in CSS is believed to be induced by persistent activation of $CD4^+$ T cells producing Th2 cytokines.[15,48] Of these, interleukin-5 (which induces eosinophil production, activation, and surveillance) seems to play a critical role.[13] Other impaired apoptosis-related molecules (eg, soluble CD95,[49] tumor necrosis factor-related apoptosis-inducing ligand receptor 3,[50] and discoidin domain receptor[51]) may contribute to the delayed eosinophil clearance seen in CSS. Infiltrating eosinophils release cytotoxic enzymes (eg, major basic protein,[52] eosinophil peroxidase,[53] and eosinophilic cationic protein[54]) and, ultimately, cause tissue injury.

In addition to interleukin-5 and other Th2 cytokines, an increase in Th1 cytokines such as TNF-α and interferon-γ has been reported in CSS. It has been hypothesized that the changing picture of CSS may respond to dynamic variations in the balance between Th1/Th2 cytokines,

ranging from Th2-mediated hypereosinophilia to Th1-induced vasculitis and granulomatous inflammation.[13]

Bacterial Antigens and Their Involvement in the Induction of Vasculitis

In 2004, a report of *in vitro* and *in vivo* studies in *Nature Medicine*[55] proposed that PR3-directed autoimmunity involved the complementary peptide of PR3 (cPR3), which is encoded by the antisense strand of the PR3 gene. Exposure to cPR3 may produce antibodies that generate anti-idiotype antibodies that are cross-reactive with PR3. PR3-encoding gene complementary sequences have been identified in microorganisms including *S. aureus*, supporting the role of infectious agents as triggers of PR3 autoimmunity via molecular mimicry. Interestingly, cotrimoxazole treatment reduces relapses in patients with WG in remission, probably by eliminating or reducing *S. aureus* in the upper airways.[56]

Kain et al[25] suggested that molecular mimicry is also the fundamental mechanism in the development of pauci-immune FNGN in patients with ANCAs. However, the antigen involved is not PR3 or MPO but LAMP-2, a heavily glycosylated type I membrane protein, which was first reported as a target of ANCAs in patients with active pauci-immune FNGN in 1995.[57] In neutrophils, LAMP-2 is located on the membranes of intracellular vesicles that contain MPO and PR3 and is also abundant on the surface of endothelial cells. LAMP-2 plays a role in antigen presentation and in the adhesion of peripheral blood mononuclear cells to the vascular endothelium.

Experimental studies are beginning to clarify some of these issues. WG is thought to begin with an aberrant cell-mediated immune response to an exogenous or endogenous antigen in the respiratory tract, which results in granuloma formation and the development of humoral autoimmunity to PR3.[58] One theory of PR3-directed autoimmunity involves the complementary peptide of PR3, which is encoded by the antisense strand of the PR3 gene.[55] Exposure of the immune system to this peptide triggers the formation of antibodies that cross-react with PR3. DNA sequences complementary to the PR3 gene have been identified in microorganisms including *S. aureus*, which supports the role of infectious agents as triggers of PR3 autoimmunity via molecular mimicry.[58]

If studies have shown that MPO-ANCAs cause pauci-immune renal vasculitis in mice,[17] how is it that anti-LAMP-2 antibodies produce a similar effect? A possible explanation is that both types of antibodies act synergistically to cause injury. On the other hand, anti-LAMP-2 antibodies might alter the function of LAMP-2 in the presentation of cytoplasmic antigens such as MPO and PR3, with the subsequent synthesis of antibodies against these proteins. In the article by Kain et al,[25] although neither epitope was homologous to MPO or PR3, the $P_{41\text{-}49}$ epitope was 100% homologous to amino acids 72 to 80 of the mature form of FimH, an adhesin located at the tip of type 1 fimbriae that is essential for the attachment of Gram-negative pathogens such as *Escherichia coli*, *Klebsiella pneumoniae,* and *Proteus mirabilis* to host epithelia. Reciprocal inhibition experiments showed that autoantibodies that recognized human LAMP-2, specifically its $P_{41\text{-}49}$ epitope, cross-reacted with FimH. Furthermore, rats immunized with recombinant FimH fusion protein developed antibodies to FimH that cross-reacted with human LAMP-2. FimH-immunized rats also developed antibodies to rat LAMP-2 and exhibited human-like pauci-immune FNGN in which 15 to 31% of glomeruli were affected by crescents; in addition, two rats developed hemorrhagic pulmonary vasculitis.

Kain et al[25] reported that 9 of 13 patients with FNGN (69%) had a microbiologically confirmed diagnosis of infection with FimH-expressing bacteria (mainly *E. coli*) during the 12 weeks before presentation. In addition, autoantibodies from these patients bound the region of human LAMP-2 that contained the cross-reactive $P_{41\text{-}49}$ epitope.

Viewed in the context of prior reports that MPO-ANCAs cause pauci-immune FNGN in mice, the findings of Kain et al[25] suggested that these antibodies might act synergistically with anti-LAMP-2 antibodies to cause injury. On the other hand, anti-LAMP-2 antibodies might alter the role of LAMP-2 in the presentation of cytoplasmic antigens such as MPO or PR3, leading to the synthesis of antibodies against these proteins.

Kain et al[25] found that more than 90% of patients with active pauci-immune FNGN had circulating anti-LAMP-2 antibodies but only around half had MPO and PR3 antibodies. A simple, accurate test for anti-LAMP-2 antibodies might, therefore, be a useful, sensitive diagnostic tool for pauci-immune FNGN. Furthermore, if it is proven that fimbriated bacteria with the relevant amino acid sequence trigger pauci-immune renal vasculitis in individuals with the required host factor, this could have far-reaching therapeutic implications: treatment of relapses with the correct antimicrobial agents could reduce the need for toxic immunosuppressants.

Pathogenesis: Uncertainties

Although the evidence for a pathogenic role of ANCAs, mainly MPO-ANCAs, is striking, various questions remain unanswered. For instance, some ANCA-negative patients fit the phenotype for MPO-ANCA-associated pauci-immune FNGN. One study found that the histological findings and prognosis in ANCA-negative pauci-immune glomerulonephritis are comparable with those of ANCA-positive disease.[59] Neutrophil cell infiltration in tissues occurs independently of circulating ANCAs in ANCA-negative disease and thus may involve unidentified autoantibodies or T cell-dependent mechanisms.[60] In WG, ANCAs remain more frequently negative in the limited form (ie, upper airways) of the disease. However, ANCAs are usually detected on progression to the systemic vasculitic stage.[61] Another counterargument is that MPO-ANCAs are not useful to monitor patients with MPO-ANCA-associated vasculitis.[62] Notably, in the aforementioned study by Kain et al,[25] of 84 patients with biopsy-proven active pauci-immune FNGN, 38 patients had MPO-ANCAs, 39 had PR3-ANCAs, and 70 patients (83%) had one or the other. However, 78 patients (93%) had

antibodies to human LAMP-2, which may well explain PR3- and MPO-ANCA negativity of some patients with typical AAV.

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